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No evidence that CD33 splicing SNP impacts the response to GO in younger adults with AML treated on UK MRC/NCRI trials

Rosemary E. Gale,¹ Teodora Popa,¹ Melissa Wright,² Naeem Khan,³ Sylvie D Freeman,³ Alan K Burnett,⁴ Nigel H Russell,⁵ Robert K Hills,² David C Linch¹

¹Department of Haematology, University College London Cancer Institute, London, UK

²Centre for Trials Research, Cardiff University, Cardiff, UK

³Department of Clinical Immunology, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK

⁴Department of Haematology, Cardiff University School of Medicine, Cardiff, UK

⁵Department of Haematology, Nottingham University Hospital NHS Trust, Nottingham, UK

Running title: CD33 splicing SNP and GO response in adult AML

Corresponding author: Rosemary E Gale,
Department of Haematology, UCL Cancer Institute,
72 Huntley Street, London WC1E 6DD, United Kingdom
Tel: +44-20-7679-6232
Fax: +44-20-7679-6222
e-mail: rosemary.gale@ucl.ac.uk

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To the editor:

Addition of the CD33-targeted immunoconjugate gemtuzumab ozogamicin (GO; Pfizer, New York, USA) has been shown to improve the response to standard induction chemotherapy and results in better long-term survival in adult patients with acute myeloid leukemia (AML).¹ The greatest impact was observed in those with favorable-risk cytogenetics, with a lesser but still significant benefit in patients with intermediate-risk cytogenetics but no benefit in those with adverse-risk cytogenetics.¹ Several studies have also demonstrated that the response positively correlates with higher levels of membrane CD33 expression on leukemic blasts.²⁻⁵ Data recently published by Lamba and colleagues further suggests that genotype at a common single nucleotide polymorphism (SNP) in the CD33 gene (rs12459419 C>T) determines response to GO in patients aged 0-29 years with de novo AML treated on the randomized phase III Children's Oncology Group Trial AAMLL0531.⁶ The SNP influences alternative splicing at CD33 exon 2 such that the C allele leads to expression of the full-length protein but the T allele is associated with increased levels of a truncated isoform lacking the external GO binding domain. The authors found that only those patients with a homozygous CC genotype (approximately 50% of patients) had a favorable response to GO, with no clinical benefit in those with either the heterozygous CT or homozygous TT genotype. The impact of GO was greatest in the CC patients with favorable risk defined as favorable cytogenetics or the presence of *NPM1* or *CEBPA* mutations. These data have important implications for the use of GO in AML, and are particularly pertinent in view of the recent approval by the US Food and Drug Administration of MYLOTARG® for treatment of AML. We therefore investigated whether similar results pertained to younger adult patients treated on United Kingdom Medical Research Council AML15 (ISRCTN17161961) and National Cancer Research Institute AML17 (ISRCTN55675535) trials. Treatment protocols and outcomes were as reported previously.^{7,8} Informed patient consent was obtained in accordance with the Declaration of Helsinki, and ethical approval for tissue use from the Wales Research Ethics Committee 3.

Genomic DNA was available from 536 of 2063 patients who were entered into different GO randomizations in these trials, and a flow chart of patients studied is shown in Supplemental Figure S1. Of these, 25 patients were randomized to receive GO in induction and consolidation and 260 in induction alone; 218 were randomized to no-GO and 33 to receive GO in consolidation alone. The latter were included in the no-GO group for the analysis as there was no evidence of a benefit for GO in consolidation.⁷ There was no difference in overall survival (OS) between those that were included or not included in our study ($P=0.06$), nor between those that were in different trials ($P=0.6$). DNA was also available from a further 184 patients scheduled (not randomized) to receive GO.

Samples were screened for the CD33 SNP using *Hae* III restriction enzyme digestion of PCR-generated amplicons (See Supplemental Data). Genotype distribution was comparable to that observed by Lamba et al⁶: 336 (47%) were CC, 319 (44%) CT and 65 (9%) TT, and the minor allele frequency was 30%. There were no differences in baseline characteristics between the genotypic groups, including age, sex, diagnosis (primary/secondary disease), WHO performance status, presenting white cell count and cytogenetics (Supplemental Table 1). The proportion of patients that received GO did not differ significantly according to genotype (52% of CC, 53% of CT, 60% of TT). The expression level of CD33 as evaluated by quantitative flow cytometry of CD33-positive blasts had previously been reported on 249 of the above patients,⁵ and the median CD33 mean fluorescence intensity was 10.7 for CC genotype patients (range, 0.2-298.1), 11.1 for CT patients (range, 0.1-134.8) and 3.8 for TT patients (range, 0.1-13.3) ($P=0.0001$ across all three groups) (Supplemental Figure S2). This finding of a similar level of expression in the CC and CT groups but a much lower level in the TT group is in accord with the data of Lamba et al.⁶

In the randomized cohort of 536 patients, the 5-year relapse-free survival (RFS) and overall survival (OS) were similar in both arms (39% vs 42% and 46% vs 47% for GO vs no-GO respectively, both $P=0.9$). There was, however a strong trend to a better outcome with GO in those patients with favorable cytogenetics (hazard ratio 0.59, 95% confidence intervals 0.30-1.14, $P=0.1$ for RFS; 0.47, 0.22-1.01, $P=0.05$ for OS). This preferential impact of GO in patients with favorable cytogenetics is in agreement with previous publications.¹

Amongst the randomized patients we found no difference in response to GO in the genotype groups. 5-year RFS for GO versus no-GO was 36% versus 42% for CC patients ($P=0.7$), 39% versus 41% for CT ($P=0.8$) and 53% versus 38% for TT ($P=0.3$) (Figure 1A). Similarly, 5-year OS was 50% versus 45% for CC patients ($P=0.3$), 40% versus 50% for CT ($P=0.1$) and 56% versus 40% for TT ($P=0.4$) (Figure 1B). When the analysis was restricted to the 87 patients with favorable cytogenetics, there was again no discernible impact of the genotype (test for heterogeneity between subgroups: Chi squared 2.0, $P=0.4$ for RFS and 2.7, $P=0.3$ for OS) (Figure 2A,B). In addition, there was no difference in the results according to the dose of GO administered (3mg/m² or 6mg/m², 152 vs 148 patients respectively) (Supplemental Figure S3).

It is difficult to explain why our results should differ so greatly from those of Lamba and colleagues. The genotype frequencies in the two populations were similar, as was the correlation between genotype and CD33 expression. Our patients were adults (age range, 13-69 years) whereas the patients in the Children's Oncology Group Trial AAML0531 were mainly children (0-29 years). It is not obvious why a difference in patient age should have such an impact, although one possibility is that multi-drug resistance due to P-glycoprotein-mediated drug efflux, which is higher in older patients⁹ and has been reported to influence response to GO,¹⁰ may mitigate against any benefit from the CC genotype in adult patients, and

this requires further investigation. The design of the randomized trials is also different, with varying schedules and doses used in the AML15 and AML17 trials investigated here, but a meta-analysis of adult patients did not suggest that these differences significantly impact outcome.¹ Our study is limited by its size (536 patients randomized), but even if the number of patients were doubled, the chance of the GO effect being significantly greater only in the CC genotype group is less than 1 in 1000. Our findings are disappointing as the ability to predict a response to GO would have a major impact on patient management and would be cost-saving. Further studies of other randomized trials of GO addition to standard therapy, both in children and in adults are warranted.

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Authorship contributions:

R.E.G. and T.P. performed genotyping. N.K., S.D.F., A.K.B. and N.H.R. provided data. M.W. and R.K.H. performed statistical analysis. R.E.G. and D.C.L. designed the study and wrote the paper. All authors reviewed and approved the manuscript.

Conflicts of interest:

The authors have no conflicts of interest to disclose.

References

1. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol.* 2014;15(9):986-996.
2. Walter RB, Gooley TA, van der Velden VHJ, et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Blood.* 2007;109(10):4168-4170.
3. Pollard JA, Loken M, Gerbing RB, et al. CD33 expression and its association with gemtuzumab ozogamicin response: Results from the randomized phase III Children's Oncology Group Trial AAML0531. *J Clin Oncol.* 2016;34(7):747-755.
4. Olombel G, Guerin E, Guy J, et al. The level of blast CD33 expression positively impacts the effect of gemtuzumab ozogamicin in patients with acute myeloid leukemia. *Blood.* 2016;127(17):2157-2160.
5. Khan N, Hills RK, Virgo P, et al. Expression of CD33 is a predictive factor for effect of gemtuzumab ozogamicin at different doses in adult acute myeloid leukaemia. *Leukemia.* 2017;31(5):1059-1068.
6. Lamba JK, Chauhan L, Shin M, et al. CD33 splicing polymorphism determines gemtuzumab ozogamicin response in de novo acute myeloid leukemia: Report from randomized phase III Children's Oncology Group Trial AAML0531. *J Clin Oncol.* 2017 Jun 23 [Epub ahead of print]
7. Burnett AK, Hills RK, Milligan D, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: Results of the MRC AML15 Trial. *J Clin Oncol.* 2011;29(4):369-377.

8. Burnett AK, Cavenagh J, Russell N, et al. Defining the dose of gemtuzumab ozogamicin in combination with induction chemotherapy in acute myeloid leukemia: a comparison of 3 mg/m² with 6 mg/m² in the NCRI AML17 Trial. *Haematologica*. 2016;101(6):724-731.
9. Leith CP, Kopecky KJ, Chen, I-M, et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia. A Southwest Oncology Group study. *Blood*. 1999;94(3):1086-1099.
10. Walter RB, Gooley TA, van der Velden VHJ, et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Blood*. 2007;109(10):4168-4170.

Figure legends

Figure 1. Outcome according CD33 genotype for SNP rs12459419 in 536 patients randomized to receive or not receive gemtuzumab ozogamicin (GO). (A) Relapse-free survival. (B) Overall survival.

Figure 2. Stratified analyses for outcome by cytogenetic risk group for patients in the GO randomization. GO, gemtuzumab ozogamicin; O-E, observed-expected; Var, variance; OR, odds ratio; CI, confidence intervals. (A) Relapse-free survival. (B) Overall survival.

Figure 1
(A)

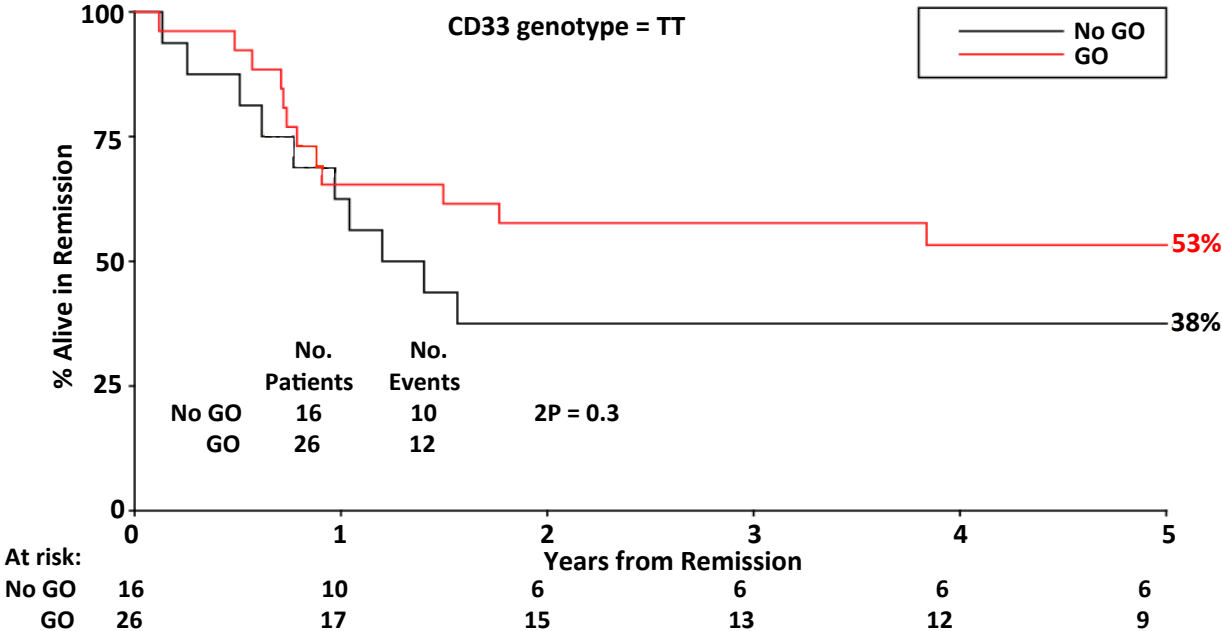
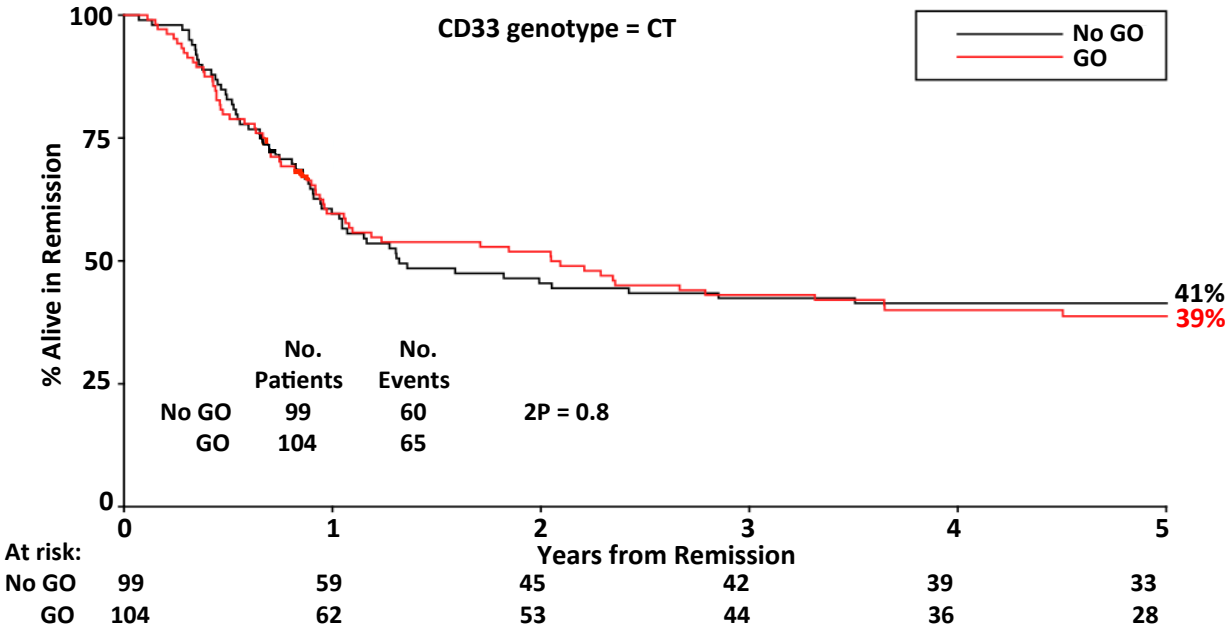
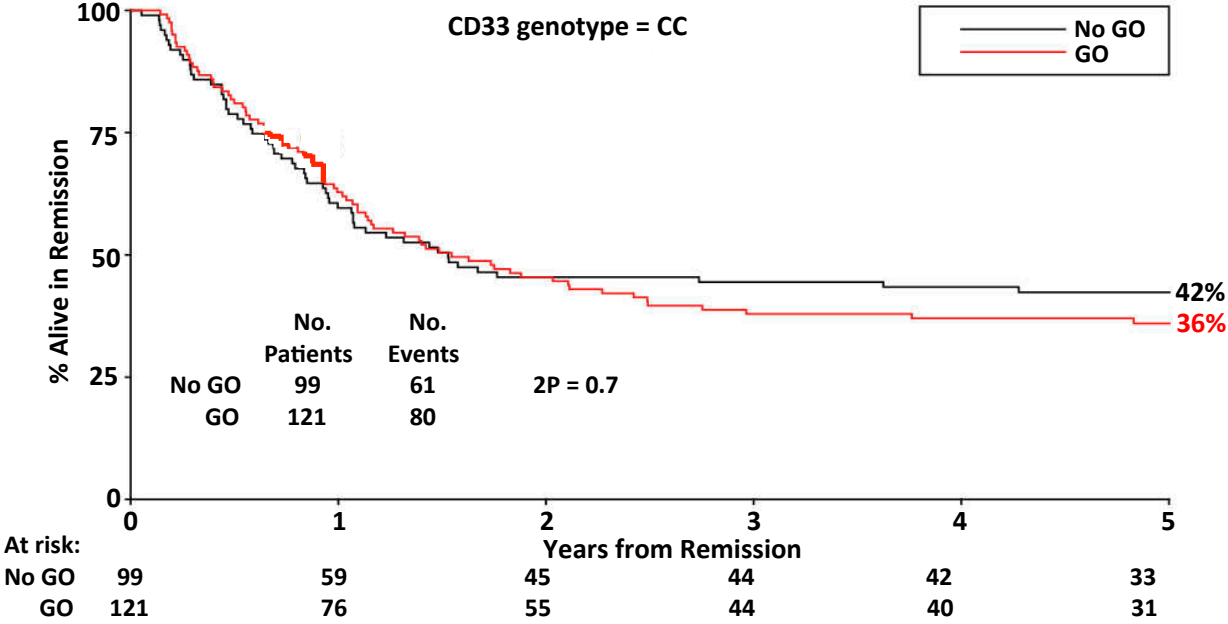


Figure 1
(B)

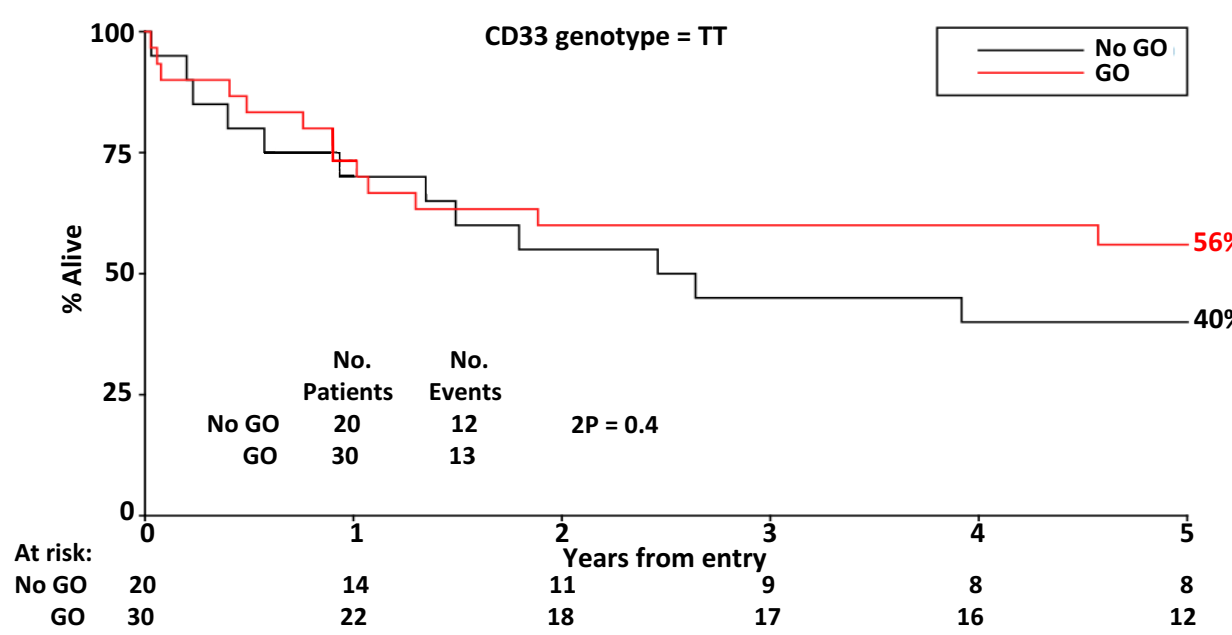
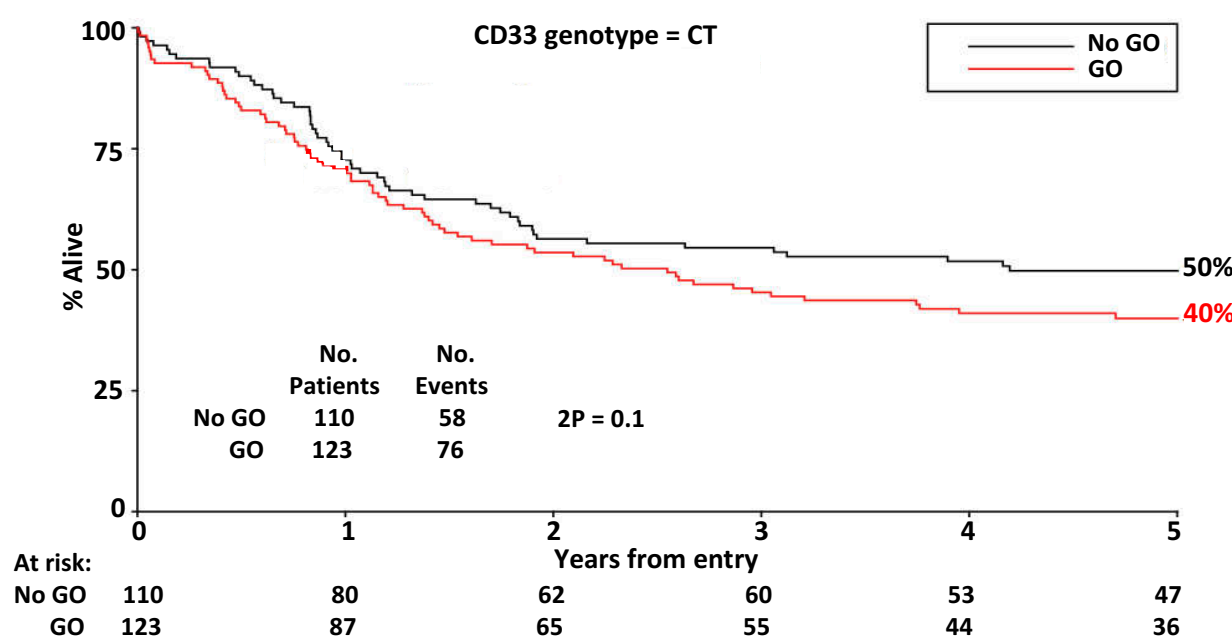
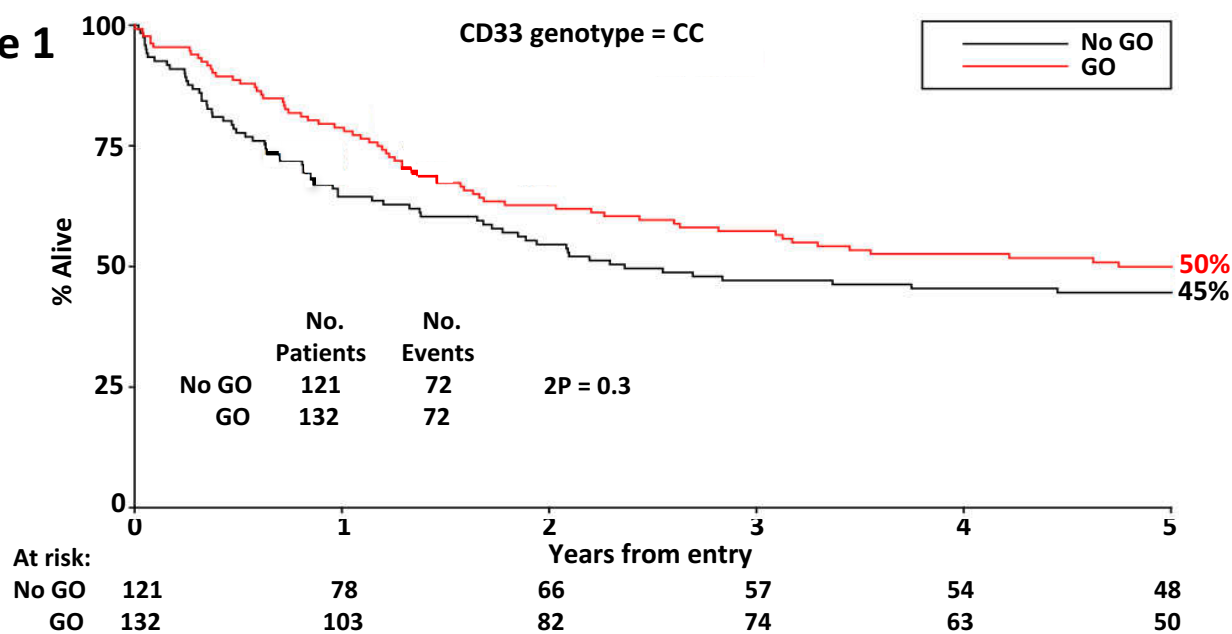


Figure 2

(A)

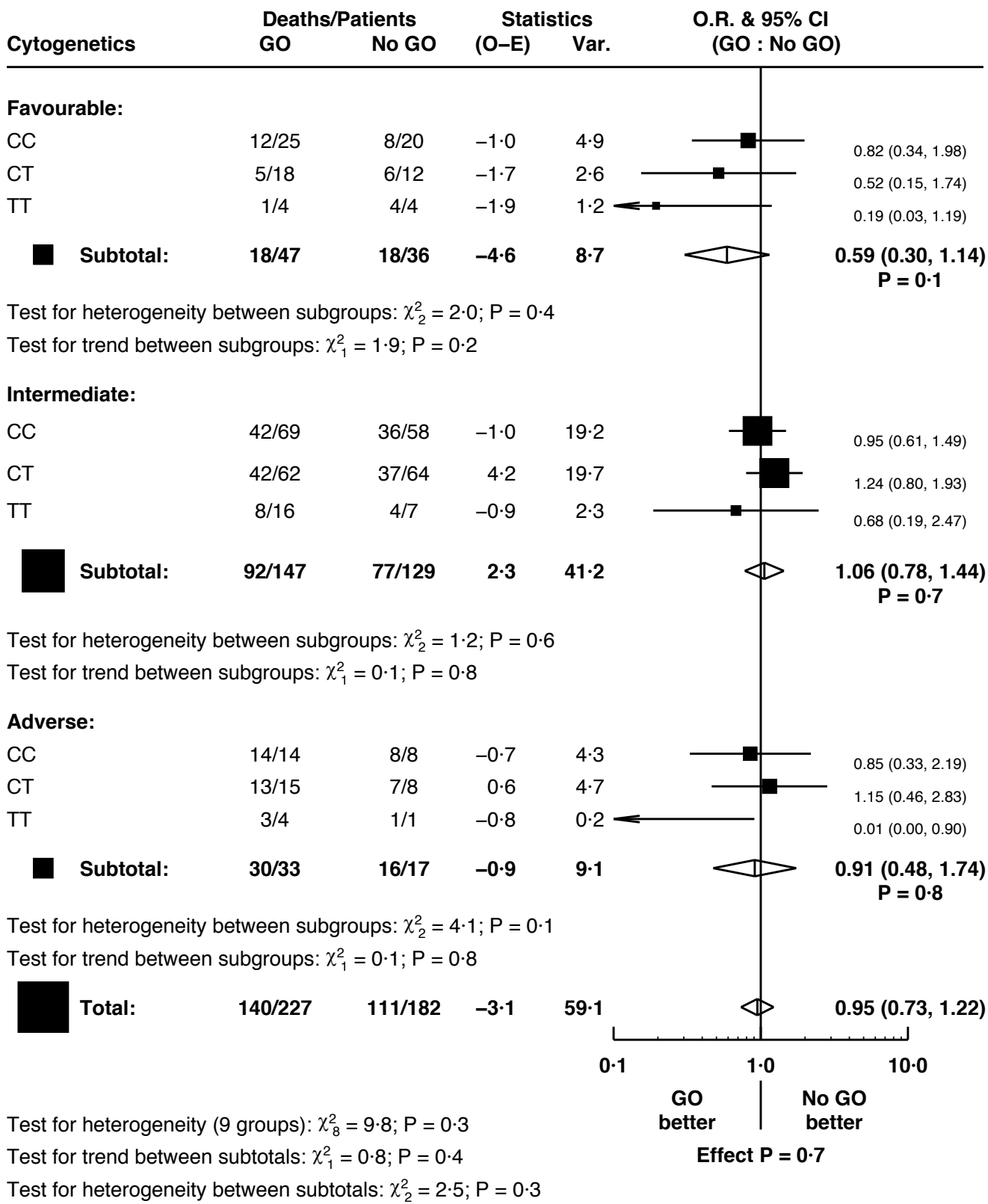
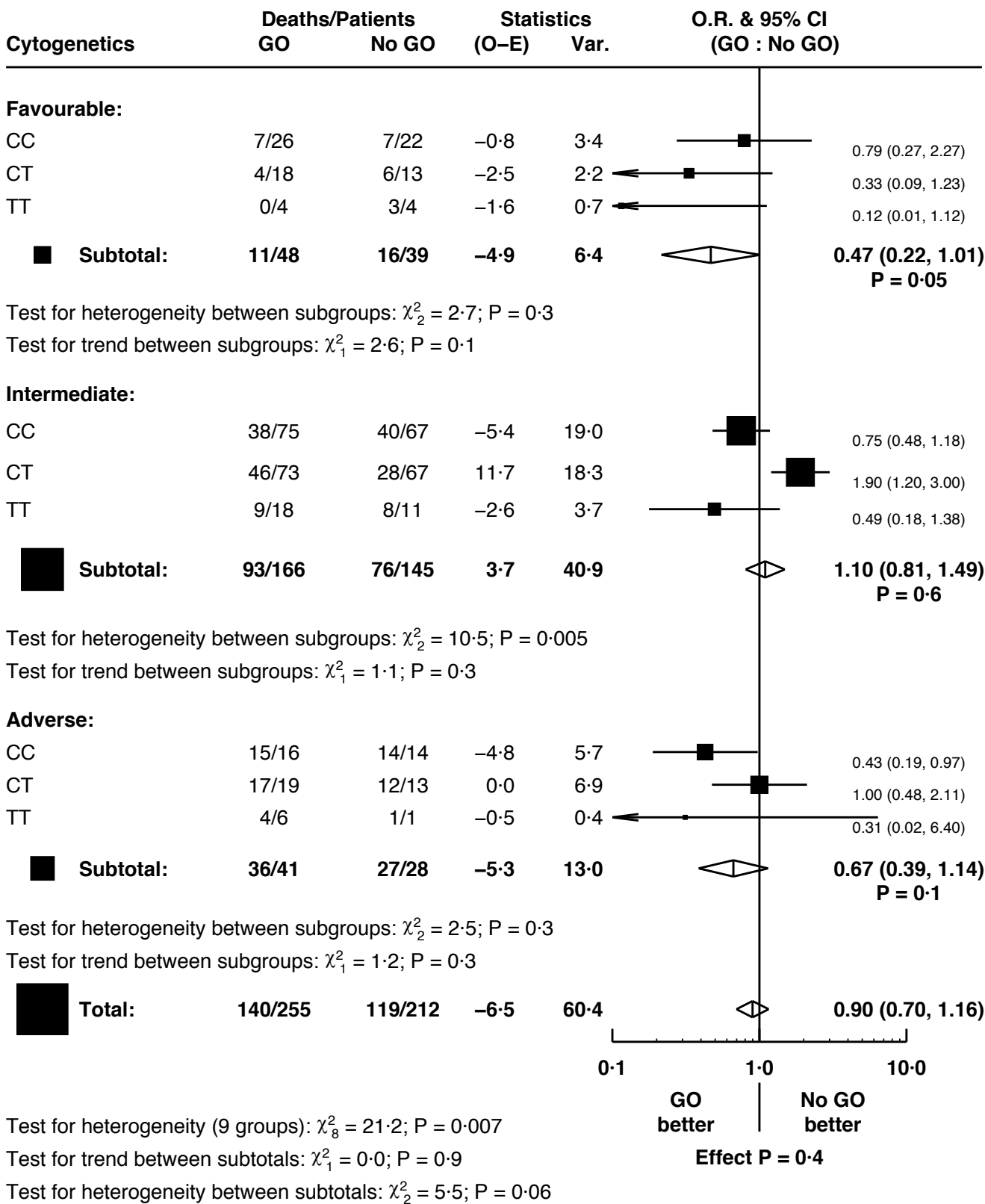


Figure 2

(B)



SUPPLEMENTAL DATA

CD33 SNP screening

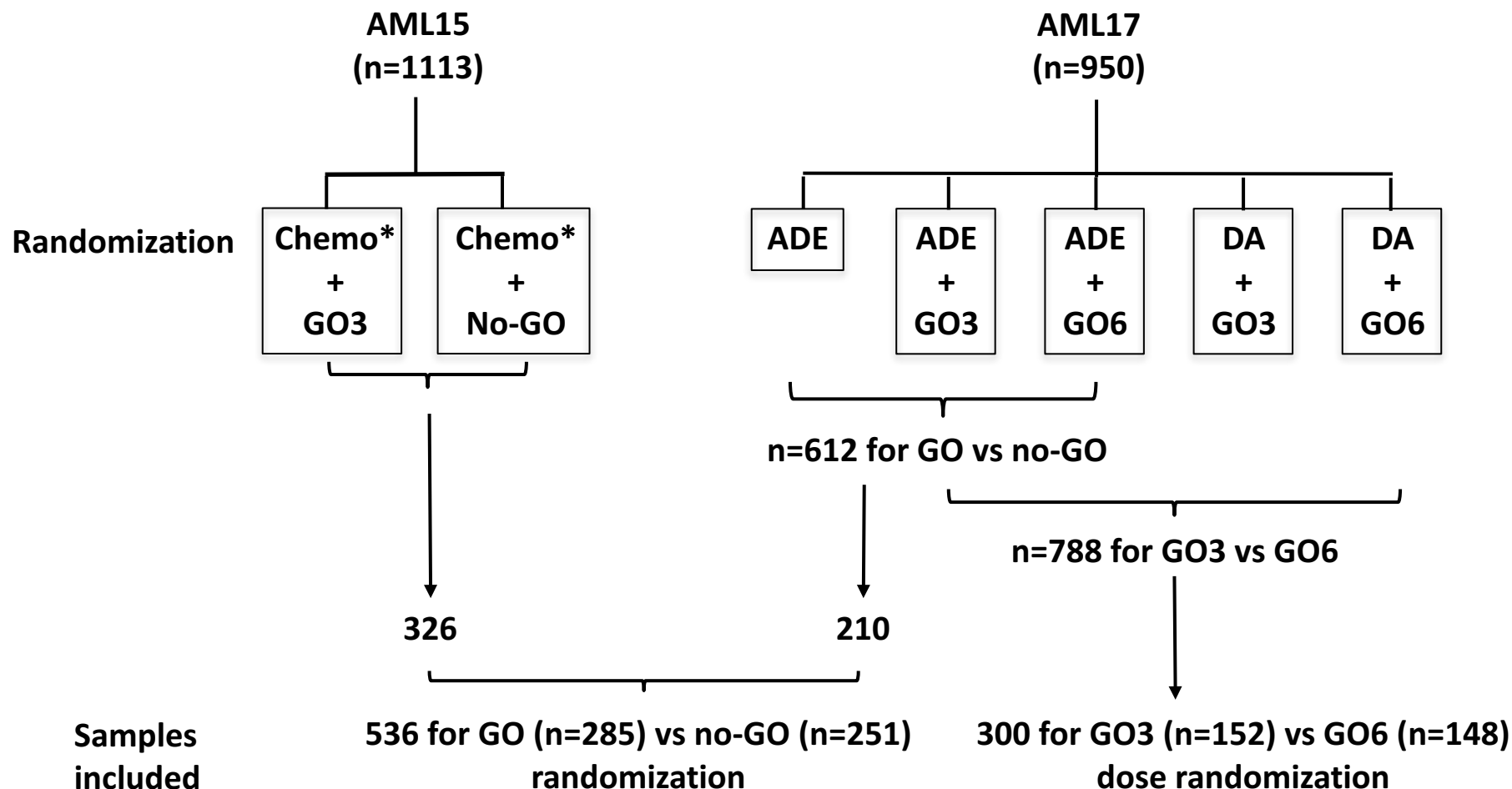
PCR products of 266bp were generated from genomic DNA using BIOTAQ DNA polymerase (Bioline, London, UK) with manufacturer's recommended conditions and primers CD33 exon 1/F (5'-CTGGAAGCTGCTTCCTCAGACATG-3') and CD33 exon 2/D (5'-GAACCAGTAACCATGAACTGGGGAGTT-3') at an annealing temperature of 66°C. Products were digested overnight with *Hae*III (New England Biolabs, Hitchin, UK) and separated on a 2% agarose gel to discriminate between the C alleles (94 + 29 + 143bp) and T alleles (94 + 172bp).

Supplemental Table 1: Demographics according to genotype for the CD33 SNP

Parameter	Total cohort	CD33 genotype			<i>P</i> CC vs not CC
		CC	CT	TT	
Patients	720	336 (47%)	319 (44%)	65 (9%)	
Median age, years (range)	48 (13-69)	48 (16-69)	49 (13-68)	49 (19-63)	0.3
Sex:					0.3
Male	395 (55%)	178 (53%)	180 (56%)	37 (57%)	
Female	325 (45%)	158 (47%)	139 (44%)	28 (43%)	
Diagnosis:					0.3
De novo	642 (89%)	304 (90%)	282 (88%)	56 (86%)	
Secondary	57 (8%)	21 (6%)	30 (9%)	6 (9%)	
High risk MDS	21 (3%)	11 (3%)	7 (2%)	3 (5%)	
WHO performance status:					0.2
0	523 (73%)	235 (70%)	239 (75%)	49 (75%)	
1	175 (24%)	90 (27%)	71 (22%)	14 (22%)	
2	13 (2%)	7 (2%)	5 (2%)	1 (2%)	
3	7 (1%)	5 (1%)	4 (1%)	1 (2%)	
4	2 (<1%)	2 (<1%)	0	0	
Median WBC, x10 ⁹ /L (range)	11.3 (0.3-456.0)	10.2 (0.8-456.0)	12.2 (0.3-430.0)	16.1 (0.9-256.3)	0.3
Cytogenetics (% of known):					0.2
Favorable	111 (17%)	57 (19%)	43 (15%)	11 (19%)	
Intermediate	436 (68%)	201 (67%)	197 (69%)	38 (64%)	
Adverse	95 (15%)	41 (14%)	44 (15%)	10 (17%)	
Unknown	78	37	35	6	
Median MFI for CD33 blasts (range)	9.4 (0.1-298.1)	10.7 (0.2-298.1)	11.1 (0.1-134.8)	3.8 (0.1-13.3)	0.1 (0.0001 across all groups)
GO given	448 (62%)	203 (61%)	198 (62%)	44 (68%)	
No GO given	272 (38%)	130 (39%)	121 (38%)	21 (32%)	
Patients in the GO vs no GO randomization:	536	253 (47%)	233 (43%)	50 (9%)	
Any SCT	299 (56%)	144 (57%)	124 (53%)	31 (62%)	
Allogeneic SCT	261 (49%)	126 (50%)	106 (45%)	29 (58%)	

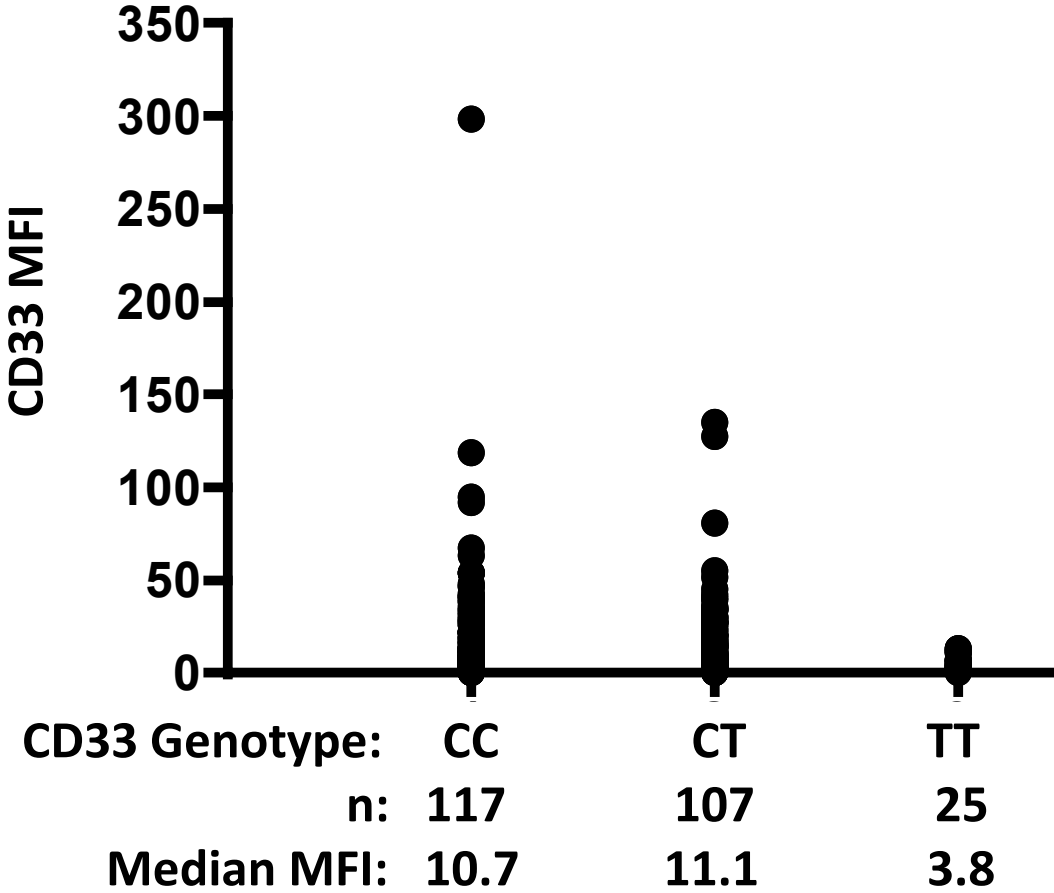
Abbreviations: MFI, mean fluorescence intensity; SCT, stem cell transplant

Supplemental Figure S1. Flow chart of patients included in the study. ADE, daunorubicin+cytarabine+etoposide; DA, daunorubicin+cytarabine; FLAG-Ida, fludarabine, cytarabine, G-CSF, idarubicin; GO, gemtuzumab ozogamicin; GO3, gemtuzumab ozogamicin at 3mg/m²; GO6, gemtuzumab ozogamicin at 6mg/m².



*Chemotherapy was ADE, DA or FLAG-Ida. FLAG-Ida resulted in a higher disease-free survival ($P=0.01$) but no significant improvement in overall survival ($P=0.2$) (Burnett et al, J Clin Oncol, 20, 3360-3368, 2013)

Supplemental Figure S2. CD33 expression levels in CD33-positive blasts according to the patients' CD33 SNP genotype. MFI, mean fluorescence intensity



Supplemental Figure 3. Overall survival according to CD33 SNP genotype in patients randomized to receive 3mg/m² or 6mg/m² GO

